Genetic Diversity and Structure of Northern Populations of the Declining Coastal Plant Eryngium maritimum

Ieviņa, Baiba; Rostoks, Nils; Syed, Naeem H.; Flavell, Andrew J.; Ievinsh, Gederts

Published in:
Proceedings of the Latvian Academy of Sciences, Section B: Natural, Exact, and Applied Sciences

DOI:
10.2478/prolas-2019-0008

Publication date:
2020

sciendo

PROCEEDINGS OF THE LATVIAN ACADEMY OF SCIENCES. Section B,

DOI: 10.2478/prolas-2019-0008

GENETIC DIVERSITY AND STRUCTURE OF NORTHERN POPULATIONS OF THE DECLINING COASTAL PLANT *ERYNGIUM MARITIMUM*

Baiba Ieviņa[1], Nils Rostoks[1,#], Naeem H. Syed[2], Andrew J. Flavell[3], and Gederts Ievinsh[1]

[1] Faculty of Biology, University of Latvia, 1 Jelgavas Str., 1, Rīga, LV–1004, LATVIA

[2] School of Human and Life Sciences, Canterbury Christ Church University, Canterbury CT1 1QU, UK

[3] Division of Plant Sciences, University of Dundee at James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK

[#] Corresponding author: e-mail: nils.rostoks@lu.lv

Contributed by Nils Rostoks

Genetic diversity among 13 northern populations of the declining coastal plant Eryngium maritimum *L. at the northernmost extent of the species distribution range was studied using retrotransposon-based SSAP molecular markers. Diversity indices varied extensively among populations; some showing extremely low diversity whereas other populations exhibited moderate amounts of genetic variation. Differentiation among populations was highly variable as well. Interestingly, differentiation among northern populations was not influenced strongly by geographic distance. Closely situated populations were often more divergent than more distant populations suggesting other factors may be responsible for genetic structuring of* E. maritimum *populations. We propose that the following genetic and environmental factors combine together in a complex relationship to mould the present genetic structure of* E. maritimum *populations in this region: (1) historic biogeographical processes; (2) local environmental conditions at each site; (3) success of sexual reproduction and proportion of clonal propagation; (4) size of the population and influence of genetic drift; (5) level of fragmentation and isolation. Lastly, we suggest that the sustainable existence of Latvian populations is seriously threatened, unless recommended conservation measures are implemented.*

Key words: *coastal plants, conservation,* Eryngium maritimum*, endangered species, genetic diversity, SSAP.*

INTRODUCTION

Coastal dune systems are particularly fragile and threatened environments characterised by highly heterogeneous climate conditions, habitat instability and high anthropogenic pressure. Despite unfavourable environmental conditions coastal dunes typically contain high species diversity in a relatively small area (Acosta *et al*., 2009). Coastal ecosystems harbour a large proportion of threatened or endangered species; therefore, they are particularly important for conservation. Sea Holly (*Eryngium maritimum* L.) is a perennial coastal species belonging to the Apiaceae family. It is distributed along coasts of Europe and adjacent parts of northern Africa comprising the Atlantic Ocean and the North, the Baltic, the Mediterranean and the Black Seas (Clausing *et al.*, 2000). Despite its wide native European distribution, populations in the northern parts of its distribution range are declining, several populations have decreased dramatically, and many populations have already become extinct in Norway, Sweden, Poland, Lithuania, Latvia and Estonia (Curle *et al*., 2007; Łabuz, 2007; Żółkoś *et al*., 2007; Aviziene *et al*., 2008; Olšauskas and Urbonienė, 2008; Minasiewicz *et al*., 2011). The species is now threatened or endangered in most northern European countries and it is included in endangered plant lists and Red Data books of several of these countries. In the northern distribution area, *E. maritimum* populations are found as fragmented patches situated along islands and the mainland (Curle *et al*., 2007).

Recently a review on *E. maritimum* was published (Iserman and Rooney, 2014), indicating continuous interest in the bi-

ology of the species. However, almost no analysis of genetic diversity of *E. maritimum* was presented in the review. Therefore, in order to fill the gap, it is necessary to point out several aspects of genetic diversity of this species in the context of its conservation status.

A goal of conservation is to preserve the maximum amount of genetic diversity of species with limited financial resources. One important aspect of this is the prioritisation of populations for conservation. The amount of intraspecific genetic variation is now widely accepted as a key parameter for practical prioritising (Frankham, 2003; Bonin *et al*., 2007). It is essential to assess the genetic diversity and genetic relationships between populations of the species to plan the appropriate conservation measures. Genetic diversity of *E. maritimum* populations has been studied locally in Norway and Poland (Curle *et al*., 2007; Minasiewicz *et al*., 2011). Also, other reports compared genetic diversity of separate individual plants across most of the species distribution range (Clausing *et al*., 2000; Kadereit *et al*., 2005). However, populations were represented typically by only one individual, resulting in an estimate of the overall genetic diversity of the species. To our knowledge no published studies on genetic diversity and population structure of *E. maritimum* populations from along the coasts of the North and Baltic Seas are available and the following study is the first to assess the genetic diversity of *E. maritimum* populations across its northern distribution range.

Genetic diversity of *E. maritium* has been previously analysed using universal nuclear ISSR and chloroplast DNA noncoding region markers (Ievina *et al.*, 2009). However, variability in these markers was too low to distinguish genetic differences among populations. We developed a new retrotransposon-based marker system for *E. maritimum*, which shows higher levels of polymorphism (Ievina *et al*., 2010). Due to retrotransposon ubiquity in the plant kingdom, extensive distribution throughout the genome, high copy numbers and heterogeneity (Flavell *et al*., 1992; Kumar and Bennetzen, 1999) retrotransposons are especially suitable for analysis of genetic structure and variation between species populations. In the present study, the retrotransposon-based SSAP molecular marker system was applied to a detailed assessment of genetic diversity and genetic relationships among and within populations of endangered species *E. maritimum* in the northern limit of its distribution range. We have used these data to both estimate the relative effects of population fragmentation, habitat type and different local environmental conditions on the genetic structure of the studied populations and to test the hypothesis that northern populations of *E. maritimum* contain distinct genetic material, primarily as a result of population fragmentation together with adaptation to different local environmental conditions.

MATERIALS AND METHODS

Plant material. Samples of *Eryngium maritimum* L. for DNA extraction were collected during summer in 2006–2008 from six Northern European countries and comprised 15 sites with 174 individual plants altogether. *E. maritimum* populations in the northern part of the species distribution range were chosen according to available data on species distribution in Northern Europe. Known present northernmost populations of *E. maritimum* were included in the analysis and consisted of populations from Saaremaa and Kihnu islands (Estonia), Gotland and Öland islands (Sweden), two sites from Latvia, Curonian Spit population in Lithuania, five sites on Polish coastline and three sites at the North-west coast in the United Kingdom. Sites that were situated less than 10 km apart (Polish coastline) and sites on small islands were designated as one population. Detailed information on samples and collection sites are given in Table 1 and Figure 1. Young leaves were collected from individual plants growing at least three metres apart to avoid sampling the same clone. Leaves were dried in silica gel until DNA extraction.

DNA extraction and SSAP analysis. Genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The retrotransposon SSAP marker system for *E. maritimum* was applied as described previously (Ievina *et al.*, 2010). Five SSAP primer combinations were selected for analysis of genetic diversity in *E. maritimum* populations: Tem2GG + PstAGG, Tem5GC + PstAGG, Tem2GG + PstCGT, Tem2GG + PstAC, Tem5GG + PstGC. SSAP analysis was performed according to the protocol of Syed and Flavell (2006). Amplification was performed using touch-down PCR conditions (Vos *et al*., 1995). Amplified SSAP fragments were resolved on a 6% 1x TBE denaturing polyacrylamide gel for 2.5 hours at 90 W and visualised by a Fuji FLA-5100 Phosphor Imager (Fuji, Japan).

Data analysis. SSAP gels were scored for the presence (1) or absence (0) of a retrotransposon insertion site. A genetic similarity matrix between accessions was calculated using the Dice coefficient with DARwin (ver 5.0) software (Perrier and Jacquemoud-Collet, unpublished data, http://darwin.cirad.fr/). After excluding any ambiguous readings 151 plants were included in the analysis. Arlequin (ver. 3.5) was used for analysis of genotype frequencies to determine distribution of genotypes in populations, as well as common and unique genotypes (Excoffier and Lischer, 2010).

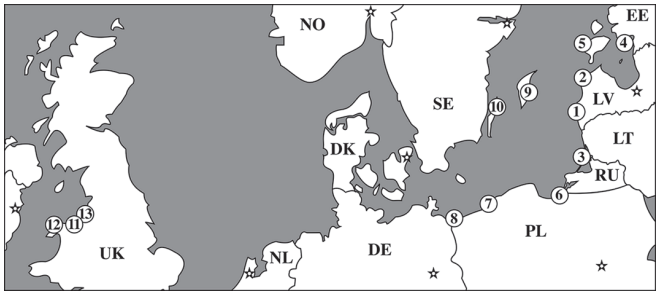

Fig. 1. Geographic location of sampled *E. maritimum* populations. 1 – 1LV, 2 – 2LV, 3 – 3LT, 4 – 4EE, 5 – 5EE, 6 – 6PL, 7 – 7PL, 8 – 8PL, 9 – 9SE, 10 – 10SE, 11 – 11UK, 12 – 12UK, 13 – 13UK.

Table 1

SAMPLE LOCATION SITES, NUMBER OF PLANTS INCLUDED IN THE SSAP ANALYSIS AND GENOTYPES SCORED

Population	Country	Collection site	Coordinates	Number of analysed plants	Number of genotypes
1LV	Latvia	Liepāja region, Ziemupe	N56°48'4" E21°4'4"	19	12
2LV	Latvia	Ventspils region, Užava	N57°14'49" E21°25'52"	32	19
3LT	Lithuania	Curonian spit, Nida	N55°22'31" E21°1'53"	10	10
4EE	Estonia	Island of Kihnu	N58°7'49" E23°57'32"	10	9
5EE	Estonia	Island of Saaremaa, Kihelkonna	N58°28'36" E21°54'59"	5	5
6PL	Poland	Vistula spit: Krynica Morska Piaski	N54°23'49" E19°28'9" N54°26'26" E19°36'30"	8	8
7PL	Poland	Dabki Lazy	N54°22'56" E16°18'3" N54°18'34" E16°11'19"	11	11
8PL	Poland	Miedzyzdroje	N53°54'51" E14°23'9"	10	9
9SE	Sweden	Island of Gotland: Folhammars naturreservat, Ljugarn Ekstakustens naturreservat, Kronvald	N57°20'50" E18°44'9" N57°17'21" E18°6'19"	8	8
10SE	Sweden	Island of Öland, Byrum	N57°13'48" E16°57'38"	14	14
11UK	United Kingdom	Wales, Prestatyn	N53°21'24" W3°23'11"	8	8
12UK	United Kingdom	Wales, Isle of Anglesey, Newborough	N53°7'58" W4°21'40"	11	11
13UK	United Kingdom	England, Formby	N53°32'27" W3°5'50"	5	5

Number of observed alleles (na), effective number of alleles (ne), Nei's gene diversity (h), and Shannon index (I) were calculated to estimate the genetic variation level using PopGene software (Yeh *et al.*, unpublished data, https://www.ualberta.ca/~fyeh/popgene.html). Differentiation of populations was measured by FST in Arlequin. Nei's analysis of gene diversity in subdivided populations (Nei, 1987) was performed in PopGene by calculating total heterozygosity (Ht) and heterozygosity within populations (Hs); genetic differentiation was further assessed with G_{ST}. Gene flow between populations was estimated using the indirect method of Wright (1931). This estimator is calculated from G_{ST} as $Nm = 0.5\ (1 - G_{ST}) / G_{ST}$. Additionally, Nei's genetic identity and genetic distance (Nei, 1972) was calculated for all pairwise combinations of populations using PopGene to examine the genetic relationship among populations.

Genetic structure of populations was estimated using Nei's (Nei, 1972) genetic distance and UPGMA clustering algorithm in PopGene. Partitioning of genetic variation among populations and individuals was performed by applying analysis of molecular variance (AMOVA) in Arlequin.

To test the hypothesis that the data is structured by an isolation by distance process (IBD) a Mantel test (Mantel, 1967) between geographic and genetic distances was performed using XLSTAT 2015 software (Addinsoft, France).

RESULTS

The isolation of LTR retrotransposons from *E. maritimum* and development of a corresponding SSAP molecular marker system have been described previously (Ievina *et al.*, 2010). Five SSAP primer combinations were applied to the 151 DNA samples in this study, producing 126 discernible bands. Twenty six of these (20.5%) were polymorphic in this data set, yielding an average of 5.2 polymorphic bands per primer combination. Overall, the marker set revealed 120 different genotypes among the 151 plants (Table 1). Eight common genotypes shared by more than one plant were identified, with the majority of these derived from Latvian population 1LV and among individuals from 1LV and 2LV. One genotype was shared among 1LV and two plants from 4EE, while another genotype was shared among Latvian populations and 8PL from Poland. In addition, one marker fragment was confined to the three UK populations. Lastly, two markers were missing in both Latvian populations, but were present in all other studied populations and two more were absent from both Swedish and Latvian populations. Such markers are, therefore, potentially useful as diagnostic tools for describing these populations.

To examine the patterns of genetic diversity, genetic variation statistics were derived for all loci. The maximum and minimum number of effective alleles was observed in populations from Lithuania (1.7425) and Latvia (1.3040), respectively (Table 2). Nei's gene diversity (h) was 0.3949 at the species level (Table 2). h and Shannon's information index (I), calculated for populations grouped according to their geographical regions (countries), indicated that diversity was the highest within the Lithuanian population (h = 0.4077, I = 0.5876) and lowest within Latvian populations (h = 0.1975, I = 0.3127). Low genetic diversity was observed also in populations from Swedish islands (h = 0.2387, I = 0.3704). *E. maritimum* populations from Estonia, Poland and the UK exhibited comparable levels of genetic diversity (h = 0.3467 – 0.3805). Even though the actual size of populations was unknown and, therefore, correlation of population size and genetic diversity could

Table 2

NEI'S GENETIC VARIATION STATISTICS FOR ALL LOCI

Region	Sample Size	na	ne	*h*	*I*
Latvia	51	1.6923	1.3040	0.1975	0.3127
Lithuania	10	1.9615	1.7425	0.4077	0.5876
Estonia	15	1.9231	1.5885	0.3467	0.5152
Poland	29	1.9615	1.6771	0.3805	0.5552
United Kingdom	24	2.0000	1.6388	0.3698	0.5497
Sweden	22	1.8077	1.3920	0.2387	0.3704
Mean		1.8910	1.5572	0.3235	0.4818
Species level	151	2.0000	1.7128	0.3949	0.5757

na, observed number of alleles; ne, effective number of alleles; *h*, Nei's (1973) gene diversity; *I*, Shannon's information index

Table 3

NEI'S ANALYSIS OF GENE DIVERSITY IN SUBDIVIDED POPULATIONS (NEI, 1987)

Region	Ht	Hs	Gst	Nm
Latvia	0.1887	0.1818	0.0363	13.2602
Lithuania	0.4077	0.4077	–	–
Estonia	0.3438	0.3000	0.1275	3.4211
Poland	0.3824	0.3239	0.1530	2.7690
United Kingdom	0.3640	0.3033	0.1666	2.5004
Sweden	0.2392	0.2161	0.0968	4.6653
Mean	0.3210	0.2888	0.1160	5.3232
Species level	0.4046	0.3223	0.2034	1.9585

Ht, total heterozygosity; Hs, heterozygosity in subpopulation; Gst, genetic differentiation; Nm, estimate of gene flow from Gst

not be estimated; visual evaluation of populations suggested that the Latvian and Swedish island populations were the smallest and these also exhibited the lowest genetic diversity (see above).

Nei's analysis of gene diversity was performed for all populations (Table 3). The total heterozygosity (Ht) and heterozygosity within populations (Hs) were 0.321 and 0.289, respectively. Diversity index among populations (G_{ST}) was 0.2034 at the species level (mean 0.116 at population level). At the regional level lowest differentiation was observed among the two Latvian (0.0363) and the two Swedish populations (0.0968). The most distinct populations were within the UK (0.1666) and Poland (0.1530). Estimate of gene flow Nm from G_{ST} was 1.9585 at the species level.

Population sub-structuring measured by F_{ST} exhibited various levels of differentiation (Table 4). Populations 1LV, 2LV, 8PL and 10SE had the highest differentiation index indicating significant divergence from all other populations, with the exception of 10SE, which was not significantly differentiated from the 5EE population. Populations 7PL, 11UK, 12UK and 9SE were significantly divergent from most of the populations. However, other populations (3LT,

Table 4

GENETIC DIFFERENTIATION OF NORTHERN EUROPEAN POPULATIONS OF *E. MARITIMUM* MEASURED BY FST

pop ID	1LV	2LV	3LT	4EE	5EE	6PL	7PL	8PL	11UK	12UK	13UK	9SE	10SE
1LV	0												
2LV	0.02482	0											
3LT	**0.39470** ***	**0.45798** ***	0										
4EE	**0.29547** ***	**0.35869** ***	0.03866	0									
5EE	**0.50764** ***	**0.60134** ***	-0.02424	0.08842	0								
6PL	**0.25903** **	**0.35265** ***	0.00511	-0.00350	0.10088	0							
7PL	**0.41905** ***	**0.48882** ***	0.01949	0.09551	**0.14627** *	-0.01687	0						
8PL	**0.07511** *	**0.16007** **	**0.23756** **	**0.21463** **	**0.39557** **	**0.1359** *	**0.23678** ***	0					
11UK	**0.44937** ***	**0.56024** ***	0.06461	**0.17627** **	0.03594	**0.14811** *	**0.22351** **	**0.33684** ***	0				
12UK	**0.32975** ***	**0.43508** ***	0.08355	**0.12892** *	**0.13670** *	0.08841	**0.17157** **	**0.26967** ***	**0.10087** *	0			
13UK	**0.33934** ***	**0.46932** **	0.15230	0.12760	0.14032	0.09694	**0.17274** **	**0.26341****	**0.17966** *	0.02696	0		
9SE	**0.47107** ***	**0.58176** ***	**0.14542** **	**0.18782** **	0.00929	**0.21210** **	**0.28924** ***	**0.41878** ***	0.06593	**0.13429** *	0.09536	0	
10SE	**0.52120** ***	**0.61267** ***	**0.14495** ***	**0.20462** ***	0.00479	**0.23191** ***	**0.30703** ***	**0.48490** ***	**0.13886** **	**0.25230** ***	**0.24289** **	**0.08721** *	0

Statistically significant fst values are in bold. F_{ST} *p* values: * $0.01 < p < 0.05$, ** $0.001 < p < 0.01$, *** $p < 0.001$

4EE, 5EE, 6PL, 13UK) exhibited low divergence (mean F_{ST} = 0.0895). Both Latvian populations 1LV and 2LV showed substantially higher divergence than other populations, with population 2LV being the most distinct among all the populations under study (mean F_{ST} = 0.425). Mean genetic differentiation (F_{ST}) over northern Europe populations was 0.226. However, when excluding the most distinct populations 1LV and 2LV, mean differentiation decreased to 0.154 at the population level and 0.109 at the regional level. All populations exhibiting the highest divergence were mutually highly distinct as well. The highest pairwise F_{ST} was for populations 2LV and 10SE (0.6127) and 2LV and 9SE (0.5818). Some population pairs exhibited very low divergence, e.g., 5EE/9SE, 5EE/10SE, 5EE/3LT, 3LT/6PL, 4EE/6PL, 6PL/7PL.

A dendrogram based on Nei's genetic distance using UPGMA clustering assigned the populations into two main groups (Fig. 2). The upper cluster contained both Latvian populations and the Polish 8PL population. The second major group contained all the other populations and consisted of two distinct subclusters. One of them included Lithuanian population 3LT, together with Polish 6PL and 7PL populations and the Kihnu island 4EE population. The other group was formed by Swedish island populations and Estonian Saaremaa island population 5EE, as well as all populations from the United Kingdom. Although some clustering according to the geographic distances between populations was apparent, the Mantel test revealed no significant correlation between genetic and geographic distances (r = –0.073, p = 0.527) (Fig. 3), indicating that genetic differentiation between remote populations is not higher than between closely located populations. Even when the three UK populations were excluded, the Mantel test showed no correlation between genetic differentiation and geographic distances of the Baltic Sea populations (r = –0.006, p = 0.967).

Analysis of molecular variance (AMOVA) showed that 73% of total genetic variation was found within populations and only 27% among populations (results not shown). When populations were grouped by geographic regions, variation within populations was 69%, variation among populations within groups was 7.6% and variation among groups was 23% (Table 5).

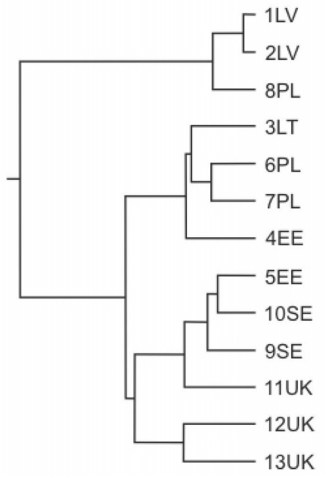

Fig. 2. UPGMA dendrogram of northern *E. maritimum* populations based on Nei's (1972) genetic distance.

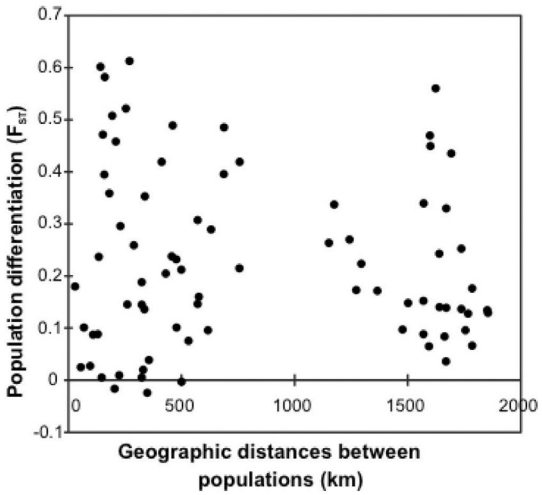

Fig. 3. Correlation between genetic and geographic distances (isolation by distance process) among northern *E. maritimum* populations. Mantel test between pairwise F_{ST} values and geographic distances between populations was performed with XLSTAT 2015.

Table 5

ANALYSIS OF MOLECULAR VARIANCE AMOVA AMONG POPULATIONS AND WITHIN POPULATIONS OF *E. MARITIMUM*

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	5	194.051	1.26516 Va	23.14
Within groups	7	57.632	0.41910 Vb	7.66
Within populations	137	518.424	3.78411 Vc	69.2
Total	149	770.107	5.46837	
Fixation indices	FSC :	0.09971		
	FST :	0.308		
	FCT :	0.23136		

DISCUSSION

In the present study, high variability of genetic diversity and differentiation across northern populations of *E. maritimum* was observed, indicating complex genetic structure of the populations. Although the overall level of differentiation among northern populations is moderate, a large number of populations exhibit low and non-significant divergence, while a few others are highly differentiated. For example, the population on Saaremaa Island is genetically similar to the populations on both Gotland and Öland islands indicating a possible gene flow among these populations. However, UK populations exhibit non-significant divergence from the Lithuanian population, but are substantially divergent from 7PL and 8PL populations from Poland.

The type of molecular markers used in the study can have a profound influence on interpretation of results. Previously, nuclear ISSR and chloroplast noncoding region markers were not able to discriminate between Latvian populations (Ievina *et al*., 2009). Although the SSAP molecular marker

system used in this study exhibited a higher level of polymorphism, identical genotypes were still found in different populations indicating that SSAP markers were not able to differentiate between closely related individuals. In addition, the dominant nature of SSAP markers may complicate statistical analysis and assessment of genetic diversity, because of the difficulty to determine allele frequencies in small populations, where Hardy–Weinberg equilibrium cannot be assumed. Nonetheless, Nei's genetic diversity (*h*) and Shannon's information index (*I*) are widely used for assessment of genetic similarity for dominant marker data.

Although Nei's indicator of gene flow Nm showed a gene flow between populations within their geographical groups, the overall species migration rate was observed to be low, which could be explained by fragmented locations of populations. Considering restricted sea currents limiting migration between Baltic and North Seas, UK populations were expected to be distinct from the Baltic Sea populations. However, this was not the case, indicating that geographical isolation is not the major contributor to the overall genetic structure of northern populations of *E. maritimum*. This assumption is further supported by non-significant isolation-by-distance (IBD) observed over the northern populations. Kadereit *et al*. (2005) reported significant correlation between geographical and genetic distances in an Atlantic cluster in three coastal plants *Cakile maritima*, *Salsola kali* and *Halimione portulacoides*, which show similar geographical distribution as *E. maritimum*, but the same study found non-significant IBD for *E. maritimum* in the Atlantic cluster. This suggests that northern populations of *E. maritimum* historically have been connected by migration. Moreover, in the same study two *E. maritimum* Mediterranean clusters showed significant correlation between genetic and geographic distances indicating that different processes are responsible for distinctiveness of *E. maritimum* populations.

The absence of IBD in this system indicates an imbalance between genetic drift and migration. These findings could be explained by the historical biogeography of the species. Our results could be explained, if the species has recently colonised its current distribution from other dispersed origins, with insufficient time to reach equilibrium by genetic drift/migration. Clausing *et al*. (2000) suggested that non-significant IBD can be explained by species presumed distribution in the Würm glacial. The species retreated into the south of Europe, because of the unfavourable environmental conditions, and recolonised northern territories from only a few colonising individuals. This suggestion was based on lower genetic distances between populations in northern territories compared to populations in the south and large differentiation between these two regions (Clausing *et al*., 2000). If this assumption is correct, it could explain the observed moderate differentiation among the northern populations.

Also, no IBD was observed in *E. maritimum* populations on the Polish coast (Minasiewicz *et al*., 2011), which could be related to historical biogeography, as well as to small spatial scale of the study. It can be assumed that populations in Northern Europe are in the evolutionary process of local adaptation after recent recolonisation of northern territories. Some populations have been more successful in adaptation to local environmental conditions, while for other populations this process is slower and may be limited due to the small size, as well as due to the limited genetic diversity for natural selection. This could at least partly explain the highly variable levels of genetic diversity and differentiation among populations.

The two Latvian populations exhibited exceptionally high divergence and low genetic diversity. While Swedish populations also exhibited comparatively lower genetic diversity, diversity and differentiation indicators for both Latvian populations were by far below the average for other populations. Moreover, Latvian and Swedish populations were highly differentiated from each other indicating distinct genetic material within each of these populations. The reasons for, or the processes sculpturing divergence of Latvian populations of *E. maritimum*, are not straightforward. There are no obvious restrictions to gene flow by sea currents among closest populations. However, considering that *E. maritimum* seeds exhibit a relatively low floating ability and short survival time in sea water (Curle *et al*., 2007), we conclude that seed dispersal and exchange of genetic material among scattered populations with comparatively large distances between them is rather limited. Rare long-distance dispersal of seeds by sea water and wind, however, may occur, producing the anomalies seen here in the relationship between geographical and genetic distances. Furthermore, Polish–Lithuanian–Estonian populations are in close proximity on the linear habitat of Baltic coast and are not significantly divergent indicating that there is another cause for the divergence of nearby Latvian populations from these.

Allele frequency analysis revealed that genetic distinctiveness of Latvian populations resulted from loss of common alleles observed in other populations rather than from accumulation of unique local alleles. Low genetic diversity and lack of common markers in Latvian populations suggests effects of genetic drift and subsequent genetic erosion. The long-term effect of genetic drift leads to loss of variability within populations and increasing differentiation among populations (Ellstrand and Elam, 1993). The considerable number of individuals with identical genotypes in the two Latvian populations points to a clonal propagation or self-fertilisation. Clonal reproduction of the species has been reported in several other studies (Curle *et al*., 2007; Aviziene *et al*., 2008; Minasiewicz *et al*., 2011); however, no studies have reported occurrence of self-fertility in this species. However, because identical genotypes were observed not only within Latvian populations, but also with 8PL and 4EE populations, clonal propagation or self-fertilisation may not be the only interpretation with the low discriminating power of SSAP marker being an alternative explanation for the observed identical genotypes. Clonal propagation could have emerged as an adaptation to unfavourable local environmental conditions observed in some *E. maritimum* popula-

tions. High precipitation and low air temperature negatively affect photosynthetic activity (Andersone *et al*., 2011) resulting in increased susceptibility to various biotic and abiotic stresses (Necajeva and Ievinsh, 2013). Cold and wet conditions often result in lower seed set as a consequence of seed abortion and slow ripening (Necajeva and Ievinsh, 2013).

High mortality of juvenile plants and high survival rates of fertile plants have been observed in *E. maritimum* populations in Norway (Curle *et al*., 2007). Moreover, elasticity matrices showed that survival of reproductive plants in these populations was more important than reproduction. Low seed production and poor germination have been reported in other northern populations (Curle *et al*., 2007; Aviziene *et al*., 2008; Minasiewicz *et al*., 2011). Low seedling establishment in populations along the Polish coast was also detected and mainly vegetative reproduction was observed (Minasiewicz *et al*., 2011) presumably to tolerate disturbances due to severe weather conditions. Regrowth of new shoots from rhizomes under unfavorable environmental conditions has been observed in several populations (Andersone *et al*., 2011) and highlights the impact of environmental factors on species reproductive success and population viability.

Environmental conditions may play an important role in determining the genetic relationship among northern populations of *E. maritimum*. Northern *E. maritimum* habitats are very diverse in respect to substrate ranging from yellow dunes, foredunes and sand beaches to grey dunes and shingle beaches. In addition, climatic conditions vary considerably among locations. Average annual temperature and precipitation, duration of vegetation period are important factors for successful sexual reproduction, seed ripening and germination, and, therefore, transfer of genetic material (Eckert, 2002; Andersone *et al.*, 2011). We suggest that unfavourable environmental conditions observed at some locations have led to limited sexual reproduction, leaving a negative impact on overall genetic diversity of the population. Several studies report low genetic diversity of *E. maritimum* in northern areas. Low molecular variation was observed in *E. maritimum* populations in Norway (Curle *et al.*, 2007). However, this study was performed using isozyme electrophoresis, which is less informative than other marker systems (de Bruin *et al*. 2003). Furthermore, low levels of genetic diversity and differentiation were found in populations on the Polish coast measured by allozyme electrophoresis (Minasiewicz *et al.*, 2011). Higher indexes of genetic diversity obtained in this study could be explained by application of a more sensitive molecular marker system. Furthermore, small size of some of these populations may be an important contributor to low genetic diversity and high divergence observed. It has been estimated that the two Latvian *E. maritimum* populations in total consist of approximately 100 individuals (Andersone *et al*., 2011). The long-term survival prospects of such populations are rather poor, as small populations are particularly vulnerable to extinction. Although clonal reproduction observed in the populations can sustain them for a long time, dry and sunny weather conditions are critical for sexual reproduction and species survival at these two sites (Andersone *et al.*, 2011).

Size and isolation of populations is an important determinant of species viability success. Decreasing numbers of suitable habitats for *E. maritimum* contribute to further fragmentation of populations, which may lead to effects of genetic drift and subsequent genetic depletion as observed in Latvian populations. Large numbers of reports of declining populations, which comprise all of the northern European distribution area, raise serious questions about survival of the species. While exact size of most of *E. maritimum* populations is unknown, a complete inventory has been done along the coasts of Poland and Lithuania (Łabuz, 2007; Aviziene *et al*., 2008). Quite a few sites in the study area consisted of only few specimens or of small groups of plants. We assume that high differentiation observed between some of populations arose from loss of genetic diversity due to genetic drift.

E. maritimum populations in northern Europe can be classified as vulnerable because of low population size and degree of isolation, and fitness. Even though the ability to reproduce vegetatively increases the persistence probability of these populations, appropriate conservation measures must be applied, including monitoring of both physiological state and genetic diversity. It is necessary to protect the existing populations *in situ* in order to preserve as much genetic variation as possible. This study shows that some of the small populations contain unique genetic material. Moreover, differentiation among some of these populations is significant. Therefore, management should aim to conserve as many of such small populations as possible, because concentrating conservation efforts only on the few larger populations would very likely result in substantial loss of genetic diversity for the species. Although many of existing populations are part of nature reserves, management should aim to incorporate all existing populations in the protected areas to reduce anthropogenic pressure by limiting access to location sites.

In order to increase genetic variation and accelerate gene flow in populations with very low genetic diversity, such as those from Latvia, *in situ* conservation should be provided. Artificial dispersal of seeds is not recommended as unfavourable environmental conditions might be detrimental to germination of seeds. Transplantation of new plants propagated through tissue culture or root fragments from the nearest populations may be a better option to increase genetic diversity in local populations and even a low differentiation can be a starting point for further evolutionary development. However, such measures must be applied with caution, because some populations are genetically differentiated, and inter-population crosses could result in reduced offspring fitness and outbreeding depression.

We conclude that some northern populations of *E. maritimum* are unique, consisting of distinct genetic material. We propose that integrity and level of intensity of several ge-

netic and environmental factors shape the present situation in each of the *E. maritimum* populations at northernmost territories of its distribution range. In particular: (1) historical processes have determined comparatively low differentiation among populations due to recent recolonisation of northern territories; (2) unfavourable environmental conditions observed at some locations have led to limited sexual reproduction having a negative impact on genetic diversity; (3) clonal propagation further contributes to low genetic diversity, (4) fragmented and isolated populations limit gene flow and exchange of genetic material among populations; and (5) small size of some populations results in limited genetic material for natural selection and local adaptation, and small populations are subject to effects of genetic drift.

ACKNOWLEDGMENTS

The study was funded by University of Latvia grants ZP-20 and ZP-59 to G. I. and N. R., respectively, a grant from Latvian Environmental Protection Fund to G. I., core funding from Scottish Executive Environment and Rural Affairs Department, and Latvian State Research Programme EVIDEnT.

REFERENCES

Acosta, A., Carranza, M. L., Izzi, C. F. (2009). Are there habitats that contribute best to plant species. *Biodivers. Conserv.*, **18**, 1087–1098.

Andersone, U., Druva-Lusite, I., Ievina, B., Karlsons, A., Necajeva, J., Samsone, I., Ievinsh, G. (2011). The use on nondestructive methods to assess a physiological status and conservation perspectives of *Eryngium maritimum* L. *J. Coastal Conserv*., **15**, 509–522.

Aviziene, D., Pakalnis, R., Sendzikaite, J. (2008). Status of red-listed species *Eryngium maritimum* L. on the Lithuanian coastal dunes. In: *Environmental Engineering. Proceedings of the 7th International Conference, May 22–28, 2008*. Vilnius Gediminas Technical University, pp. 22–28.

Bonin, A., Nicole, F., Pompanon, F., Miaud, C., Taberlet, P. (2007). Population adaptive index: A new method to help measure intraspecific genetic diversity and prioritize populations for conservation. *Conserv. Biol*., **21**, 697–708.

de Bruin, A., Ibelings, B. W., Van Donk, E. (2003). Molecular techniques in phytoplankton research: From allozyme electrophoresis to genomics. *Hydrobiologia*, **491**, 47–63.

Clausing, G., Vickers, K., Kadereit, J. W. (2000). Historical biogeography in a linear system: Genetic variation of Sea Rocket (*Cakile maritima*) and Sea Holly (*Eryngium maritimum*) along European coasts. *Mol. Ecol*., **9**, 1823–1833.

Curle, M., Stabbetorp, O. E., Nordal, I. (2007). *Eryngium maritimum*, biology of a plant at its northernmost localities. *Nord. J. Bot*., **24**, 617–628.

Eckert, C. G. (2002). The loss of sex in clonal plants. *Evol. Ecol*., **15**, 501–520.

Ellstrand, N. C., Elam, D. R. (1993). Population genetic consequences of small population size: Implications for plant conservation. *Annu. Rev. Ecol. Evol. Syst*., **24**, 217–242.

Excoffier, L., Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Mol. Ecol. Resour*., **10**, 564–567.

Flavell, A. J., Dunbar, E., Anderson, R., Pearce, S. R, Hartley, R., Kumar, A. (1992). Ty1-*copia* group retrotranspozons are ubiquitous and heterogenous in higher plants. *Nucleic Acids Res*., **20**, 3639–3644.

Frankham, R. (2003). Genetics and conservation biology. *Comptes Rendus Biol*., **326**, 22–29.

Ievina, B., Rostoks, N., Ievinsh, G. (2009). Analysis of genetic diversity of Latvian population of Sea Holly *Eryngium maritimum* L. [Jūrmalas zilpodzes *Eryngium maritmum* L. Latvijas populācijas ģenētiskās daudzveidības analīze] *Latvijas Veģetācija*, 18, 13–24 (in Latvian).

Ievina, B., Syed, N. H., Flavell, A. J., Ievinsh, G., Rostoks, N. (2010). Development of retrotransposon-based SSAP molecular marker system for study of genetic diversity in sea holly (*Eryngium maritimum* L.) *Plant Genet. Resour*., **8**, 258–266.

Iserman, M., Rooney, P. (2014). Biological flora of British Isles: *Eryngium maritimum. J. Ecol*., **102**, 789–821.

Kadereit, J. W., Arafeh, R., Somogyi, G., Westberg, E. (2005). Terrestrial growth and marine dispersal? Comparative phylogeography of five coastal plant species at a European scale. *Taxon*, **54**, 861–876.

Kumar, A., Bennetzen, J. L. (1999). Plant retrotransposons. *Annu Rev. Genet*., **33**, 479–532.

Łabuz, T. A. (2007). Evaluation of past and present Sea Holly (*Eryngium maritimum*) habitats on Polish coastal dunes. *Acta Univ. Latv*., **723**, 99–114.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res*., **27**, 209–220.

Minasiewicz, J., Borzyszkowska, S., Żółkoś, K., Bloch-Orłowska, J., Afranowicz, R. (2011). Population genetic structure of the rare species *Eryngium maritimum* L. (Apiaceae) on the Gulf of Gdańsk: Implications for conservation and management. *Biodiv. Conserv*., **24**, 39–48.

Necajeva, J., Ievinsh, G. (2013). Seed dormancy and germination of an endangered coastal plant *Eryngium maritimum* (Apiaceae). *Estonian J. Ecol*., **62**, 150–161.

Nei, M. (1972). Nei's original measures of genetic identity and genetic distance. *Amer. Nat*., **106**, 283–292.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*, **70**, 3321–3323.

Nei, M. (1987). *Molecular Evolutionary Genetics*. 1st Ed. Columbia University Press, New York. 512 pp.

Olšauskas, A. M., Urbonienė, R. (2008). State of *Eryngium maritimum* L. population on the Curonian Spit coastal dunes. *Environ. Res. Eng. Man*., **2**, 69–74.

Syed, N. H., Flawell, A. J. (2006). Sequence-specific amplification polymorphisms (SSAPs): A multi-locus approach for analyzing transposon insertions. *Nat. Protoc*., **1**, 2746–2752.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res*, **23**, 4407–4414.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, **16**, 97–159.

Żółkoś, K., Afranowicz, R., Bloch-Orlowska, J., Koziel, K. (2007). Distribution and the resources of Sea Holly (*Eryngium maritimum* L.) on the western shore of the Gulf of Gdansk. *Biodiv. Res. Conserv*., **5**, 55–60.

Received 20 November 2018
Accepted in the final form 2 January 2019

IZZŪDOŠĀS PIEKRASTES SUGAS *ERYNGIUM MARITIMUM* ZIEMEĻU POPULĀCIJU ĢENĒTISKĀ DAUDZVEIDĪBA UN STRUKTŪRA

Pētījumā analizēta izzūdošās piekrastes sugas *Eryngium maritimum* areāla ziemeļu daļā izvietoto 13 populāciju ģenētiskā daudzveidība, izmantojot uz retrotranspozoniem balstītus SSAP molekulāros marķierus. Ar daudzveidību saistītie parametri būtiski atšķīrās starp populācijām, dažām bija raksturīga ārkārtīgi zema daudzveidība, bet citās populācijās bija novērojams mērens ģenētiskās mainības līmenis. Arī diferenciācija starp populācijām bija ļoti variabla. Interesanti, ka diferenciācija starp ziemeļu populācijām nebija būtiski atkarīga no ģeogrāfiskā attāluma. Tuvu izvietotas populācijas bieži bija atšķirīgākas nekā attālākas populācijas, parādot citu faktoru ietekmi uz *E. maritimum* populāciju ģenētisko struktūru. Var pieņemt, ka sekojoši ģenētiskie un vides faktori kopā veido sarežģītās mijiedarbības, kas nosaka *E. maritimum* populāciju ģenētisko struktūru šajā reģionā: (1) vēsturiskie bioģeogrāfiskie procesi; (2) lokālie vides apstākļi katrā atradnē; (3) dzimumvairošanās veiksme un klonālās vairošanās proporcija; (4) populācijas lielums un ģenētiskā dreifa ietekme; (5) fragmentācijas un izolācijas līmenis. Visbeidzot, var uzskatīt, ka, bez īpašu saglabāšanas pasākumu ieviešanas, Latvijas populācijas ilgtspējīga pastāvēšana ir nopietni apdraudēta.